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KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,  
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ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,  
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,  
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For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: ACETYLATED HMGB1 PROTEIN

(57) Abstract: An isolated multiply acetylated protein HMGB1; or a variant or fragment thereof, or a polynucleotide encoding therefor.



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# INTERNATIONAL SEARCH REPORT

PCT/IB 03/05718

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/47 C12N5/10 A61K38/17 G01N33/50 C07K16/18  
C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, EMBASE, WPI Data, SEQUENCE SEARCH, MEDLINE, EMBL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>STERNER R ET AL: "STUDIES OF ACETYLATION AND DEACETYLATION IN HIGH MOBILITY GROUP PROTEINS IDENTIFICATION OF THE SITES OF ACETYLATION IN HIGH MOBILITY GROUP PROTEIN 1"</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 254, no. 22, 1979, pages 11577-11583, XP002279281</p> <p>ISSN: 0021-9258</p> <p>the whole document</p> <p style="text-align: center;">--- -/--</p>	1-55

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
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- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*G\* document member of the same patent family

Date of the actual completion of the international search

12 May 2004

Date of mailing of the international search report

17/06/2004

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

PCT/IB 03/05718

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE EMBL 'Online!  "HMGB1 protein and mRNA"  retrieved from EBI  Database accession no. NP_002119  XP002279285  the whole document  -&amp; DATABASE EMBL 'Online!  retrieved from EBI  Database accession no. NM_002128  XP002282370  the whole document</p>	1-55
Y	<p>POLEVODA B ET AL: "The diversity of  acetylated proteins"  GENOME BIOLOGY 2002 UNITED KINGDOM,  vol. 3, no. 5, 2002, pages 0006.1-0006.6,  XP002279282  ISSN: 1465-6906  the whole document</p>	1-55
Y	<p>GARDELLA STEFANIA ET AL: "The nuclear  protein HMGB1 is secreted by monocytes via  a non-classical, vesicle-mediated  secretory pathway."  EMBO REPORTS,  vol. 3, no. 10, October 2002 (2002-10),  pages 995-1001, XP002279283  ISSN: 1469-221X (ISSN print)  the whole document</p>	1-55
Y	<p>SCAFFIDI PAOLA ET AL: "Release of  chromatin protein HMGB1 by necrotic cells  triggers inflammation"  NATURE, MACMILLAN JOURNALS LTD. LONDON,  GB,  vol. 418, no. 6894,  11 July 2002 (2002-07-11), pages 191-195,  XP002261326  ISSN: 0028-0836  the whole document</p>	1-55
Y	<p>THOMAS J O ET AL: "HMG1 and 2, and  related 'architectural' DNA-binding  proteins"  TIBS TRENDS IN BIOCHEMICAL SCIENCES,  ELSEVIER PUBLICATION, CAMBRIDGE, EN,  vol. 26, no. 3, 1 March 2001 (2001-03-01),  pages 167-174, XP004229458  ISSN: 0968-0004  the whole document</p>	1-55
X	<p>WO 99/59609 A (VANDER WAY LIMITED  ;BARTORELLI ALBERTO (IT))  25 November 1999 (1999-11-25)  claim 7</p>	44

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## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00/47104 A (PICOWER INST MED RES) 17 August 2000 (2000-08-17) abstract ---	33-40, 44 .
P, X	BONALDI TIZIANA ET AL: "Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion." EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 22, no. 20, 15 October 2003 (2003-10-15), pages 5551-5560, XP002279284 ISSN: 0261-4189 (ISSN print) the whole document ---	1-55
P, X	WO 03/026691 A (BIANCHI MARCO ;MANFREDI ANGELO (IT); SAN RAFFAELE CENTRO FOND (IT)) 3 April 2003 (2003-04-03) the whole document -----	1-55

# INTERNATIONAL SEARCH REPORT

PCT/IB 03/05718

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Although claims 33-37, 41, 43-45, 47-55 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.2

Present claims 12-19, 23-37, and 42-55 relate to a compounds defined by reference to a desirable characteristic or property, namely modulation of acetylated HMGB1.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the antibodies and antisense molecules.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

## INTERNATIONAL SEARCH REPORT

PCT/IB 03/05718

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9959609	A	25-11-1999	IT MI981094 A1	19-11-1999
			AT 211391 T	15-01-2002
			AU 4363199 A	06-12-1999
			DE 69900756 D1	28-02-2002
			DE 69900756 T2	13-03-2003
			DK 1079849 T3	22-04-2002
			WO 9959609 A2	25-11-1999
			EP 1079849 A2	07-03-2001
			ES 2171086 T3	16-08-2002
			JP 2002515445 T	28-05-2002
			PT 1079849 T	28-06-2002
WO 0047104	A	17-08-2000	US 6303321 B1	16-10-2001
			AU 3698300 A	29-08-2000
			CA 2359926 A1	17-08-2000
			EP 1165110 A2	02-01-2002
			JP 2003520763 T	08-07-2003
			WO 0047104 A2	17-08-2000
			US 2003017155 A1	23-01-2003
			US 2003113323 A1	19-06-2003
			US 2003143194 A1	31-07-2003
			US 6468533 B1	22-10-2002
			US 2002102609 A1	01-08-2002
WO 03026691	A	03-04-2003	IT MI20011986 A1	25-03-2003
			WO 03026691 A2	03-04-2003